Scientific Opinion on objections of a Member State to a notification (Reference C/NL/13/01) for the placing on the market of the genetically modified carnation SHD-27531-4 with a modified colour, for import of cut flowers for ornamental use, under Part C of Directive 2001/18/EC from Suntory Holdings Limited

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Abstract: The European Commission asked the Scientific Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) to address the objections of Cyprus to the placing on the market of the genetically modified (GM) carnation SHD-27531-4. The GM carnation cut flowers, with a modified petal colour, are intended to be imported and distributed in the European Union for ornamental use only. The EFSA GMO Panel responded to the objections of Cyprus, taking into account the limited intended use of carnation SHD-27531-4 and the information available. First, the EFSA GMO Panel concludes that the propagation of carnation SHD-27531-4 by individuals cannot be excluded. However, should this occur, carnation SHD-27531-4 would not show any potential for increased survival, fitness or weediness compared with its parental line. Second, the EFSA GMO Panel is of the opinion that the potential spread of pollen of the GM carnation SHD-27531-4 by Lepidoptera to wild Dianthus species cannot be eliminated but is highly unlikely to occur and, if it did occur, it is very unlikely that viable hybrids would be produced, survive and result in adverse environmental effects. Third, considering the very low potentials for hybridisation and/or seed production of (GM) carnations, the EFSA GMO Panel concludes that plant-to-plant gene transfer of the introduced genes is very unlikely and, if it did occur, it is unlikely to result in viable seed production leading to adverse environmental effects.

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SCIENTIFIC OPINION

Scientific Opinion on objections of a Member State to a notification (Reference C/NL/13/01) for the placing on the market of the genetically modified carnation SHD-27531-4 with a modified colour, for import of cut flowers for ornamental use, under Part C of Directive 2001/18/EC from Suntory Holdings Limited[1]

EFSA Panel on Genetically Modified Organisms (GMO)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The European Commission asked the Scientific Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) to address the objections of Cyprus to the placing on the market of the genetically modified (GM) carnation SHD-27531-4. The GM carnation cut flowers, with a modified petal colour, are intended to be imported and distributed in the European Union for ornamental use only. The EFSA GMO Panel responded to the objections of Cyprus, taking into account the limited intended use of carnation SHD-27531-4 and the information available. First, the EFSA GMO Panel concludes that the propagation of carnation SHD-27531-4 by individuals cannot be excluded. However, should this occur, carnation SHD-27531-4 would not show any potential for increased survival, fitness or weediness compared with its parental line. Second, the EFSA GMO Panel is of the opinion that the potential spread of pollen of the GM carnation SHD-27531-4 by Lepidoptera to wild *Dianthus* species cannot be eliminated but is highly unlikely to occur and, if it did occur, it is very unlikely that viable hybrids would be produced, survive and result in adverse environmental effects. Third, considering the very low potentials for hybridisation and/or seed production of (GM) carnations, the EFSA GMO Panel concludes that plant-to-plant gene transfer of the introduced genes is very unlikely and, if it did occur, it is unlikely to result in viable seed production leading to adverse environmental effects.

KEY WORDS

carnation, cut flower, *Dianthus* sp., Directive 2001/18/EC, environment, petal colour, import

[1] On request from the European Commission, Question No EFSA-Q-2014-00446, adopted on 22 October 2014.

[2] Panel members: Salvatore Arpaia, Andrew Nicholas Edmund Birch, Andrew Chesson, Patrick du Jardin, Achim Gathmann, Jürgen Gropp, Lieve Herman, Hilde-Gunn Hoen-Sorteberg, Huw Jones, József Kiss, Gijs Kleter, Martinus Lovik, Antoine Messéan, Hanspeter Naegeli, Kaare Magne Nielsen, Jaroslava Ovesna, Joe Perry, Nils Rostoks and Christoph Tebbe. Correspondence: gmo@efsa.europa.eu

[3] Acknowledgement: The Panel wishes to thank the members of its standing Working Group on Environmental Risk Assessment (ERA) of GMOs for the preparatory work on this scientific opinion and EFSA staff: Andrea Gennaro, Sylvie Mestdagh and Yann Devos for the support provided to this scientific opinion.

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SUMMARY

In accordance with the procedure laid down in Directive 2001/18/EC[4], the European Commission asked the Scientific Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) to address the objections raised by Cyprus to notification C/NL/13/01 on the genetically modified (GM) carnation SHD-27531-4. The GM carnation cut flowers, with a modified petal colour, are intended to be imported and distributed in the European Union for ornamental use only.

In delivering the present scientific opinion, the EFSA GMO Panel considered information provided in notification C/NL/13/01, the report on the initial environmental risk assessment carried out by the competent authority of the Netherlands, relevant scientific publications and the experience gained in assessing GM carnations with similar traits.

Considering the scope of notification C/NL/13/01 and the available information, the EFSA GMO Panel concludes that:

1. The propagation of carnation SHD-27531-4 (e.g. rooting) by individuals cannot be excluded. However, should this occur, carnation SHD-27531-4 would not show any potential for increased survival, fitness or weediness compared with its parental line.
2. The potential spread of pollen of the GM carnation SHD-27531-4 by Lepidoptera to wild *Dianthus* species cannot be eliminated but is highly unlikely to occur and, if it did occur, it is very unlikely that viable hybrids would be produced, survive and result in adverse environmental effects.
3. Taking into account the very low potentials for hybridisation and/or seed production of (GM) carnations, plant-to-plant gene transfer of the introduced genes is very unlikely and, if it did occur, it is unlikely to result in viable seed production leading to adverse environmental effects.

[4] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. OJ L 106, 17.4.2001, p. 1–39. Available online: http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32001L0018

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION AND EFSA

In July 2013, the European Commission received the notification (Reference C/NL/13/01) on the genetically modified (GM) carnation SHD-27531-4, together with the report on the initial environmental risk assessment carried out by the competent authority of The Netherlands, lead Member State. The scope of notification C/NL/13/01 submitted by Suntory Holdings Limited covers the import, distribution and retailing in the EU of carnation SHD-27531-4 cut flowers for ornamental use only.

In accordance with Directive 2001/18/EC[5], the notification was then transmitted to the competent authorities of other Member States. A number of which raised comments and objections during the statutory 60-day period. The notifier, Suntory Holdings Limited, provided the Member States with additional information in response to the comments and objections raised during the 60-day period. However, one Member State (i.e. Cyprus) maintained objections which could not be solved during the statutory 105-day period, in which case the European Commission is required to follow the procedure of Article 18(1) of Directive 2001/18/EC.

In accordance with Article 18(1), the European Commission consults the European Food Safety Authority (EFSA), for a scientific opinion in response to the three objections raised by Cyprus. In the present scientific opinion, the EFSA GMO Panel addresses these objections in the light of the scope of notification C/NL/13/01 and according to the principles described in its guidance documents for the risk assessment of GM plants and derived food and feed (EFSA, 2010, 2011). For its evaluation, the EFSA GMO Panel considered information provided in notification C/NL/13/01, the report on the initial environmental risk assessment carried out by the competent authority of The Netherlands, relevant scientific publications and the experience gained in assessing GM carnations with similar traits.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

EFSA is requested, in accordance with Article 28 of Directive 2001/18/EC, to provide a scientific opinion whether there is any scientific reason to believe, as suggested by the objecting Member States (i.e. Cyprus), that:

1. human aided propagation of genetically modified carnation line SHD-27531-4 cannot be excluded;
2. the risk of potential spread of pollen by Lepidoptera insects in the endemic species *Dianthus* occurring in Cyprus cannot be eliminated;
3. negligible potential for gene transfer would exist even if all imported cut flowers were kept outside for the duration of their use.

[5] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. OJ L 106, 17.4.2001, p. 1–39. Available online: http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32001L0018

ASSESSMENT

1. Introduction

Carnation SHD-27531-4 petals are purple resulting from the expression of two new genes encoding dihydroflavonol 4-reductase and flavonoid 3′5′ hydroxylase. Carnation SHD-27531-4 also contains a mutated herbicide tolerance gene, *SuRB* (*als*), which encodes an acetolactate synthase (ALS) variant protein used to facilitate the selection of the transformed shoots *in vitro* during the genetic transformation process.

Upon request of the European Commission (see the Section above "Terms of reference"), the Scientific Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) addressed the objections raised by Cyprus in the light of the scope of notification C/NL/13/01 (genetically modified (GM) carnation cut flowers for ornamental use) and in accordance with the principles described in its guidance documents for the risk assessment of GM plants and derived food and feed (EFSA GMO Panel, 2010, 2011). For its evaluation, the EFSA GMO Panel considered information provided in notification C/NL/13/01[6], the report on the initial environmental risk assessment carried out by the competent authority of the Netherlands, relevant scientific publications and the experience gained in assessing GM carnations with similar traits (see EFSA, 2006, 2008, on GM carnations Moonlite™ and Moonaqua™, respectively).

2. Evaluation of the relevant scientific data

Considering the scope of notification C/NL/13/01, there will be very limited environmental exposure with respect to viable plant parts of the GM carnation. The environmental risk assessment (ERA) is mainly concerned with the consequences of exposure through: (1) unintended release into the environment of GM carnations obtained by vegetative multiplication, (2) pollen dispersal from GM cut flowers to other carnations and wild relatives, and (3) dispersal of seeds produced by GM cut flowers and possible progeny. These routes of exposure are therefore considered by the EFSA GMO Panel in the present opinion.

The EFSA GMO Panel assessment of the GM carnation SHD-27531-4 below is structured following the three objections of Cyprus.

2.1. First objection of Cyprus: "Human aided propagation of genetically modified carnation line SHD-27531-4 cannot be excluded"

Carnation is the common name of *Dianthus caryophyllus* (i.e. cultivated carnation). Although this *Dianthus* sp. does not spread vegetatively through organs such as bulbs, stolons or rhizomes, the cultivated carnation can be vegetatively propagated to produce plants for cut flower production. Cuttings are taken from "mother plants/stems", which are continually pruned to produce a large number of vegetative cuttings from axillary buds. These cuttings are rooted in conditions of high humidity after treatment to encourage root growth. Rooted plants may be planted in soil or grown hydroponically, and are kept for one to two years. Flowers are produced in flushes, beginning three to five months after rooted cuttings are planted.

Carnation SHD-27531-4 plants are imported as cut flowers and thus have no roots and only occasional vegetative buds. The cut stems with vegetative shoots could be propagated by rooting or by micro-propagation. The latter is a multiplication technique applied in the laboratory which requires specific expertise and appropriate material for successful tissue culture. The EFSA GMO Panel is of the opinion that this technique is unlikely to be used by individuals (e.g. amateur gardeners) to propagate GM carnations. However, the GM carnation could be propagated by rooting and released into the environment (e.g. gardens); therefore, the EFSA GMO Panel has considered the consequences of such potential releases.

[6] Notification C/NL/13/01 – Sections A, B and C.

In general, carnation varieties compete poorly outside their cultivated environment. In addition, carnation varieties do not show weedy characteristics. The notifier also provided data[7] on 22 morphological characteristics of the GM carnation SHD-27531-4 compared with its parental (recipient) line from two pot trials in Australia in 2010 (see also Section 2.2). Significant differences were observed between the GM carnation and its parental line for 7 out of the 22 morphological characteristics studied (number of internodes per stem, leaf length, petal length, number of viable anthers, filament length, number of filaments and number of petals per flower), but these were not observed consistently throughout the two pot trials. However, the differences are not related to characteristics associated with increased invasiveness or survival, except in the presence of sulfonylurea herbicides. Therefore, the EFSA GMO Panel concludes that the differences observed are unlikely to affect the overall fitness of the GM carnation compared with its parental line.

Moreover, the EFSA GMO Panel is not aware of any scientific reports of increased spread and establishment of (GM) carnations or of any change in survival capacity, including overwintering (COGEM report[8]; EFSA, 2006, 2008). In addition, *D. caryophyllus* has been imported into all EU countries as a garden ornamental plant and cut flower for many decades and EFSA is not aware of any reports of feral populations that have established outside of cultivation.

Considering the scope of notification C/NL/13/01 and the available information, the EFSA GMO Panel agrees with Cyprus that the propagation of carnation SHD-27531-4 (e.g. rooting) by individuals cannot be excluded. However, should this occur, carnation SHD-27531-4 would not show any potential for increased survival, fitness or weediness compared with its parental line.

2.2. Second objection of Cyprus: "The risk of potential spread of pollen by Lepidoptera insects in the endemic species *Dianthus* occurring in Cyprus (i.e. *Dianthus cyprius* and *Dianthus strictus* var. *troodi*) cannot be eliminated"

Members of the genus *Dianthus*, including wild and domesticated species, are fairly diverse, as their origins range from southern Russia to Alpine regions of Greece and the Auvergne mountains of France. *Dianthus* spp. are adapted to the cooler Alpine regions of Europe and Asia, and are also found in Mediterranean coastal regions. *D. caryophyllus* is a widely cultivated ornamental plant in Europe, both in glasshouses and outdoors (i.e. in Italy and Spain) and is occasionally naturalised in some Mediterranean countries but appears to be restricted to the coastal Mediterranean regions of Greece, Italy, Sicily, Corsica and Sardinia (Tutin et al., 1993).

Wild *Dianthus* species exhibit a diversity of phenotypes, exploiting niches in a wide geographical range in Europe (Tutin et al., 1993). In addition, they have very close associations with specific pollinator species owing to their floral fragrance (Jürgens et al., 2003). In this scientific opinion, the EFSA GMO Panel paid particular attention to species of *Dianthus* that are endemic to Cyprus. *D. cyprius*, which is endemic to northern Cyprus, is mainly found on high limestone cliff faces, whereas *D. strictus* var. *troodi* is found mainly in the Troodos mountains of central Cyprus.

The majority of *Dianthus* spp. are self-sterile because the stigma is not receptive to pollen until one week or more after anthers have shed pollen. Cultivated carnations require hand pollination to set seed (Bird, 1994). As a result of the long history of use of vegetative propagation and selection for flower characteristics, the carnation produces only a negligible amount of pollen and, consequently, seed set is low or absent (Galbally and Galbally, 1997). The quantity and quality of pollen varies with the cultivar (Kho and Baer, 1973; Galbally and Galbally, 1997). Carnation pollen is heavy and sticky and has low viability. Wind plays only a small role in pollen dispersal (Office of the Gene Technology Regulator, 2005). In the wild, cross-pollination of *Dianthus* spp. is by insect pollinators, in particular by Lepidoptera, which have probosces of sufficient length to reach the nectaries at the base of the flowers. However, the GM carnation has double flowers with a high density of petals. These obstruct

[7] Notification C/NL/13/01 – Attachment A11.

[8] Available online: http://www.cogem.net/index.cfm/en/publications/publicatie/advisory-report-import-distribution-and-retail-of-cut-flowers-with-modified-flower-colour-gm-carnation-shd-27531-4

insect pollinators from probing the flowers to reach the nectaries and therefore discourage insect pollinator activity and limit the amount of pollen they collect and transfer to other flowers.

The notifier also provided data[9] on morphological characteristics from two pot trials in Australia in 2010, as already mentioned in Section 2.1. In one of the two trials, no viable anthers were observed in flowers. In the other pot trial, the notifier measured pollen diameter and pollen viability by exclusion of the dye acetocarmine and by germination on pollen germination medium. Carnation SHD-27531-4 produced significantly fewer filaments, fewer viable anthers and shorter filaments than the parental line. The notifier concluded that pollen of the GM carnation is "far less viable than pollen from the parental line".

The EFSA GMO Panel is therefore of the opinion that the likelihood of a lepidopteran pollinator collecting pollen from GM carnation cut flowers or from an illegally propagated and cultivated GM carnation, and transferring it to wild *Dianthus* species occupying these more remote ecological niches, is extremely unlikely.

Carnation flowers have been propagated and cultivated throughout Europe for many years and EFSA is not aware of any reports that this has resulted in hybridisation with wild species. Where plant breeders and horticulturalists have attempted to hybridise different *Dianthus* species, this has resulted in very few viable or fertile hybrids being produced and, generally, methods such as embryo capture and tissue culture have had to be used to produce hybrids. In addition, monitoring reports[10] from similar GM carnation transformation events did not indicate any cross-pollination (EFSA, 2006, 2008).

As discussed in Section 2.1 and above, there is no indication that the characteristics of the GM carnation would confer any selective advantage or adaptation if hybridised with wild *Dianthus* species in Cyprus.

In conclusion, the EFSA GMO Panel is of the opinion that the potential spread of pollen of the GM carnation SHD-27531-4 by Lepidoptera to wild *Dianthus* species cannot be eliminated but is highly unlikely to occur and, if it did occur, it is very unlikely that viable hybrids would be produced, survive and result in adverse environmental effects.

2.3. Third objection of Cyprus: "Negligible potential for gene transfer would exist even if all imported cut flowers were kept outside for the duration of their use"

As discussed in Section 2.2, the notifier provided data on morphological characteristics indicating that viable seed production on cut flowers is very unlikely and has not been observed to date with carnation SHD-27531-4, most likely because of its limited life time (i.e. three weeks) in comparison with the time needed for complete seed development (i.e. five weeks).

The EFSA GMO Panel also considered the possibility of natural exchange of genetic material with another carnation variety, *D. caryophyllus* L., as well as with wild *Dianthus* species (see Section 2.2). The majority of *Dianthus* spp. are self-sterile because the stigma is not receptive to pollen until one week or more after anthers have shed pollen. Cultivated carnations require hand pollination to set seed (Bird, 1994). As a result of the long history of use of vegetative propagation and selection for flower characteristics, the carnation produces only a negligible amount of pollen and, consequently, seed set is low or absent (Galbally and Galbally, 1997). The quantity and quality of pollen varies with the cultivar (Kho and Baer, 1973; Galbally and Galbally, 1997). Carnation pollen is heavy and sticky and has low viability. Wind plays only a small role in pollen dispersal (Office of the Gene Technology Regulator, 2005). In the wild, cross-pollination of *Dianthus* spp. is by insect pollinators, in particular by Lepidoptera, which have probosces of sufficient length to reach the nectaries at the base of the flowers. However, the GM carnation has double flowers with a high density of petals. These obstruct

[9] Notification C/NL/13/01 – Attachment A11
[10] For example, 2013-2014 monitoring report published at http://www.bvl.bund.de/EN/Home/homepage_node.html

insect pollinators from probing the flowers to reach the nectaries and therefore discourage insect pollinator activity and limit the amount of pollen they collect and transfer to other flowers. This severely limits the probability of spontaneous hybridisation between the GM carnation and other cultivated carnations and so the likelihood of seed set and the establishment of viable hybrids is considered to be very low.

The very low potentials for hybridisation and/or seed production lead the EFSA GMO Panel to conclude that plant-to-plant gene transfer of the introduced genes is very unlikely and, if it did occur, it is unlikely to result in viable seed production leading to adverse environmental effects.

3. Conclusion

Considering the scope of notification C/NL/13/01 and the available information, the EFSA GMO Panel responds to each of the three objections of Cyprus as follows:

1. The propagation of carnation SHD-27531-4 (e.g. rooting) by individuals cannot be excluded. However, should this occur, carnation SHD-27531-4 would not show any potential for increased survival, fitness or weediness compared with its parental line.
2. The potential spread of pollen of the GM carnation SHD-27531-4 by Lepidoptera to wild *Dianthus* species cannot be eliminated but is highly unlikely to occur and, if it did occur, it is very unlikely that viable hybrids would be produced, survive and result in adverse environmental effects.
3. Taking into account the very low potentials for hybridisation and/or seed production of (GM) carnations, plant-to-plant gene transfer of the introduced genes is very unlikely and, if it did occur, it is unlikely to result in viable seed production leading to adverse environmental effects.

The EFSA GMO Panel also refers to the post-market environmental monitoring (PMEM) plan[11] provided by the notifier, as it addresses some of the concerns raised by Cyprus. The PMEM plan includes (1) a questionnaire for the European importers and operators, including questions on unexpected adverse effects and "illegal growing"; (2) consumer feedback; (3) the survey carried out among botanists to report on any wild populations or unusual *Dianthus* hybrids that might be found during their routine survey work; (4) the consultation of existing networks (e.g. national plant protection services); (5) a literature review; and (6) the screening of websites, databases, etc. The EFSA GMO Panel is of the opinion that the scope of the PMEM plan proposed by the notifier is in line with the restricted intended use of carnation SHD-27531-4.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the European Commission, dated 23 May 2014, to the EFSA Executive Director asking for the risk assessment of the genetically modified carnation SHD-27531-4 from Suntory Holdings Limited for the purpose of import, under Part C of Directive 2001/18/EC (notification C/NL/13/01).
2. Acknowledgement letter, dated 16 June 2014, from EFSA to the European Commission.

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[11] Notification C/NL/13/01 – Section D

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